"On the Synthesis of Fats accompanying Absorption from the Intestine." By BENJAMIN MOORE, M.A., D.Sc., Johnston Professor of Bio-chemistry at University College, Liverpool. Communicated by Professor C. S. SHERRINGTON, F.R.S. Received June 15,—Read June 18, 1903.

(From the Physiological Laboratory, University College, Liverpool.)

The experiments here recorded form a continuation of work previously carried out by the author in conjunction with D. P. Rockwood* and with W. H. Parker.†

In the earlier papers it was shown that the bile possesses solvent properties for both free fatty acids and soaps, which taken in conjunction with the hydrolytic action of the pancreatic juice upon fats renders the view highly probable that all the fats of the food are absorbed, not as an emulsion but in soluble form as fatty acids and soaps. Such a view yields an easy explanation of the conjoint action of the bile and pancreatic juice in fat absorption, and of the defective uptake of fat when either fluid is absent from the intestine.

It also supplies an important function for the bile, and explains why the circulation of the biliary acids occurs.

These results have since been confirmed and in certain respects extended by Pflüger‡, who also supports the view that all the fat of the food is taken up from the intestine in soluble form.

The experiments described in this present communication were designed chiefly with the object of studying the subsequent changes which take place in these absorbed soluble constituents of fat digestion.

It is known from the experiments of Munk§ that, even when free fatty acids are taken in as food, neutral fat is the chief fatty constituent present in the lymph of the thoracic duct, and this is also the case when neutral fat is being taken up from the intestine.

This observation clearly demonstrates that the fatty acids and soaps formed in the intestine are synthesized back into neutral fat before the thoracic duct is reached, but no clear experimental proof is in existence as to where along the channel of absorption this synthesis occurs, nor in what manner it takes place, that is to say, as to whether it is carried out by an intracellular enzyme, or is dependent on and inseparable from cells lying somewhere along the path of absorption.

* 'Roy. Soc. Proc.,' vol. 60, 1897, p. 438; 'Journ. of Physiology,' vol. 21, 1897, p. 58.

† 'Roy. Soc. Proc.,' vol. 68, 1901, p. 64.

‡ 'Arch. f. die ges. Physiologie,' vol. 82, 1900, pp. 303, 381; vol. 85, 1901, p. 1; vol. 88, 1902, pp. 299, 431; vol. 90, 1902, p. 1.

§ 'Virchow's Arch.,' vol. 80, 1880, p. 17; vol. 95, 1884, p. 452.

It is true that the change is currently believed to take place in the columnar cells of the intestine, but a review of the experimental evidence on which this statement rests will suffice to show that the proof is incomplete, and hence the subject has here been more rigorously investigated.

The chief facts for the view that the synthesis occurs in the intestine are, the histological appearance presented by the columnar cells when taken during fat absorption and examined by the usual micro-chemical tests for fats; secondly, the naked-eye appearance of the mesenteric lacteals and microscopic examination of their contents during fat absorption; and, thirdly, the supposed action of extracts of intestinal mucous membrane in synthesizing, *in vitro*, neutral fats from solutions of soap and glycerine.

The objections to these supposed proofs are, that the histological and naked-eye appearances taken to be characteristic of fat in the columnar cells and mesenteric lacteals might equally well be given by free fatty acid in suspension, and hence supply no proof of a synthesis of neutral fat, since no chemical examination of the fatty matters present at these stages in fat absorption have hitherto been made so far as the author is aware. In the second place, the proofs given of the synthesis of neutral fat *in vitro* from soap and glycerine by intestinal cells are based upon incomplete analyses, and, as will be shown later, are erroneous.

It may be stated at once, however, that the present investigation has shown that the synthesis does occur in the intestinal mucosa, or, to put the matter more rigorously, before the mesenteric lacteals have been reached, but that it has been impossible to imitate this action *in vitro* either by detached cells or cell-free extracts.

There are two places along the path of the absorbed fatty material from intestine to thoracic duct at which the lymph comes into intimate relationship with cells, and at which accordingly chemical changes might be expected to occur. These situations are the intestinal villus and the mesenteric lymphatic gland, and the experimental method of procedure suggests itself of examining the chemical composition of the fatty matter in these situations, and before and after passing through them. This method has been employed in the present investigation, and in so doing the percentage of neutral fat and of free fatty acids have both been *directly* determined in ethereal extracts of the intestinal mucosa, and lymph of the mesenteric lymphatic vessels obtained during fat absorption.

The lymph in the mesenteric lacteals during fat absorption has not previously been examined chemically as to its content in neutral fat and free fatty acid, probably from the difficulty experienced in collecting it in sufficient quantity from the exceedingly narrow vessels.

It is difficult to wash the intestinal mucous membrane free from attached globules of fat which lie between the villi, and hence to be certain that all the fat submitted to analysis has been obtained from the interior of the villi and columnar cells, and so the results recorded below cannot be taken as quantitatively accurate although they do undoubtedly show that a considerable amount of synthesis has occurred in the villi, and that the synthesis is in progress and not in that state of completion which is found in the lymph of the mesenteric lacteals.

It is hence fortunate that it was found possible to obtain lymph from the small mesenteric lymph vessels in sufficient quantity to make analyses of both neutral fat and free fatty acid, and so prove conclusively that the latter is practically absent by the time the lymph is leaving the intestinal wall.

It was found quite impossible to introduce a cannula into these lymphatic vessels, and hence the lymph was obtained by cleansing the mesenteric surface, opening the lymphatic with a fine pointed pair of scissors, carefully avoiding accompanying blood vessels, and allowing the lymph to escape on to the mesenteric surface. The fluid was then collected in wide capillary tubes, by capillarity or suction, until a sufficient amount had been obtained for the necessary analyses.

A second method of investigating the action of the various cells which the lymph, containing the fatty constituents, encounters on its path of absorption, is to prepare these tissues, or extracts of them free from cells, and test *in vitro* whether such preparations exert any synthetic action.

This method was also employed with extracts of intestinal mucous membrane and lymphatic glands, and as the results differed essentially from those of previous observers who had used this method, but without making so complete analyses of the supposed synthesized products, pancreatic extracts were also similarly tested.

Since it was also thought that the absence of synthesis of neutral fat shown by the experiments might be due to the lack of a supply of energy by the cells or enzymes contained in them, an attempt was made in a certain number of the experiments to supply a source of energy by the addition of glucose to the soap and glycerine of the other experiments. But here also the result remained negative and it may be stated that throughout no appreciable synthesis of neutral fat from its constituents was ever obtained.

One positive result observed, which does possess a certain degree of physiological importance, was that all three types of extract, but especially that of the pancreas, possess a marked power of setting free fatty acid from the soap employed.

A protective action would be exercised in the body in this manner against the appearance of the highly poisonous soaps in the circulation.

C. A. Ewald* was the first observer who stated that the fresh mucous membrane of the small intestine is capable *in vitro* of synthesizing neutral fat from a mixed solution of soap and glycerine, and recently Hamburger† has published a similar result obtained with the mucous membrane of the large intestine. Both the above observers, however, relied upon the difference in weight between the total ethereal extract, and the weight of fatty acid, as shown by titration with standard alkali, of the ethereal extract, for giving the amount of neutral fat, and did not make direct determinations by saponification with caustic alkali of the amount of neutral fat. Here it is believed by the present writer that they fell into an error, for had such an indirect method been used in the experiments recorded below, similar results to those of Ewald and of Hamburger would have been obtained in many cases. The direct determination by saponification shows, however, that the difference between total ethereal extract and free fatty acid is due not to neutral fat, but to soap dissolved by the ether.‡

Other observers have described synthesis, by extracts of intestinal mucous membrane, and several other tissue extracts, of such esters as ethyl-butyrate,§ and monobutyrin,‖ but no detailed account of these experiments need be given since the conditions of synthesis and hydrolysis of such esters are probably widely different from those applying to the triglycerides.¶

I.—*Experiments on the composition of the lymph of the mesenteric lymphatic vessels during fat absorption.*

The lymph was obtained by the method described above from the lacteals of anæsthetised dogs, at a period of 5—7 hours, subsequent to feeding on olive oil.

The ethereal extract obtained from the lymph was evaporated to dryness, dissolved in hot alcohol and titrated with decinormal sodic hydrate solution using phenol-phthalein as an indicator.

From the amount of standard sodic hydrate used the percentage of free fatty acid in the ethereal extract was calculated.

* 'Arch. f. Physiol. u. Anat., Physiol. Abth.,' Suppt. vol., 1883, p. 302.

† 'Arch. f. Physiol.,' 1900, p. 433.

‡ Hamburger attempted in his experiments to remove such a source of error, by making a control to which soap was added after digestion, but the amount of soap dissolved appears to vary so as to make this procedure ineffectual, and reliable results can only be obtained by a direct estimation by saponification of the neutral fat.

§ Kastle and Loevenhart, 'American Chemical Journal,' 1900, vol. 24, p. 491; Loevenhart, 'American Journal of Physiology,' 1902, vol. 6, p. 331.

‖ Hanriot, 'Comptes rendus de la Société de biologie,' 1901, p. 70.

¶ See Lewkowitsch, 'Journal of Society of Chemical Industry,' 1903, vol. 22 No. 2.

The neutral alcoholic solution thus obtained was next evaporated down to a small volume, a measured volume (usually 10 cc.) of standard alcoholic potash solution was added, and the mixture boiled for 20—30 minutes, in a small flask fitted with a reflux tube, in order to insure complete saponification of all the neutral fat present.* Finally the contents of the flask were accurately neutralised by standard hydrochloric acid, with phenol-phthaleïn as indicator. The difference in value between alkali added before boiling and acid required afterwards then supplied the necessary *datum* for calculating the percentage of neutral fat.

Expt. 1.—A dog, weighing 12 kilos., was fed with 100 grammes of olive oil at 9.30 A.M. and anæsthetised at 3.30 P.M., when the lacteals were exposed, and seventeen drawn-out tubes were filled with milk-white chyle by opening the lacteals.

	Grammes.
Weight of lymph	= 1·2990
,, total ethereal extract..	= 0·0618
,, free fatty acid	= 0·0028
,, neutral fat	= 0·0564
Percentage of free fatty acid	= 4·7
,, neutral fat	= 95·3

Expt. 2.—Weight of dog = 8·6 kilos., fed with 50 grammes of olive oil at 9.30 A.M.; chyle collected as before at 4.30 P.M., and twenty-three tubes charged with the fluid.

The lymph was weighed and analysed with the following results:—

	Grammes.
Weight of lymph.....	= 0·9550
,, ethereal extract.....	= 0·1052
,, free fatty acid.....	= 0·0042
,, neutral fat.....	= 0·1031
Percentage of free fatty acid	= 3·9
,, neutral fat	= 96·1

Expt. 3.—Weight of dog = 12·4 kilos., fed at 9 A.M. with 100 grammes of olive oil; chyle collected at 3 P.M. by a fine pipette into a porcelain capsule, and then weighed and analysed.

	Grammes.
Weight of lymph	= 1·8712
,, ethereal extract.....	= 0·1450
,, free fatty acid	= 0·0042
,, neutral fat	= 0·1326
Percentage of free fatty acid	= 3·1
,, neutral fat	= 96·9

Hence in these experiments practically all the fatty matter (upwards of 95 per cent.) is present in neutral fat.

* Köttstorfer's method, as described in Sutton's 'Volumetric Analysis,' 8th Ed., p. 402. Preliminary experiments to test the method with pure neutral olein gave practically theoretical values.

II. *Experiments on the Composition of the Fatty Constituents present in the Intestinal Mucosa during Fat Absorption.*

The animals used in the previous set of experiments were killed immediately after the lymph had been collected, and the entire small intestine was removed, cut open longitudinally and thoroughly washed in running water to remove as completely as possible all adherent fat.

The intestine was next stretched out, mucous surface upward, upon a clean glass plate and the mucous membrane rubbed off with the back of a knife.

The pulpy mucous membrane thus obtained was weighed and extracted, first with a mixture of alcohol and ether (1 alcohol to 3 of ether) and then with ether alone.

The solvents were decanted off, the solutions mixed and evaporated to dryness. The dried residue was next extracted with dry ether, filtered, and the ethereal extract evaporated to dryness.

The residue was weighed, and in it the amount of free fatty acid and neutral fat were determined by the methods already described in connection with the previous series of experiments.

Expt. 1.—Total weight of moist mucous membrane = 31·7 grammes; weight of total ethereal extract = 1·1524 grammes; weight of free fatty acid = 0·1802 gramme; weight of neutral fat = 0·9722 gramme; percentage of free fatty acid = 15·7; percentage of neutral fat = 84·3.

Expt. 2.—Weight of moist mucous membrane = 14·4 grammes; weight of total ethereal extract = 0·8074 gramme; weight of free fatty acid = 0·2904 gramme; weight of neutral fat = 0·5303 gramme; percentage of free fatty acid = 35·4; percentage of neutral fat = 64·6.

In these experiments the percentage of free fatty acid is much higher than in the lymph of the mesenteric lacteals, showing that the process of synthesis is in progress, and not yet complete.

III. *Experiments on the Action of Pancreatic, Lymphatic and Intestinal Cells, and of Cell-free Extracts of such Cells, upon Solutions of Soap and Glycerine.*

The tissues and extracts used in these experiments were obtained from the cat, dog, ox or pig, and similar effects were in all cases obtained.

In the case of the intestinal mucous membrane, the intestine taken from a freshly-killed animal was cut open longitudinally from end to end and then thoroughly washed either in a stream of running tap water or with 0·75 per cent. solution of sodium chloride.

It may be stated that no difference was ever found throughout the entire series of experiments in the action of extracts made with distilled water and those prepared with normal saline.

The mucosa was scraped off the inner surface of the intestine by rubbing with the back of a knife, and the soft uniform mass so obtained was gathered in a heap and chopped up on a glass plate. It was then transferred to a mortar and rubbed up alone or mixed with fine sand.

Portions were weighed out and treated with definite amounts of the extractives used, in an incubator at 36° C. for varying times.*

When the action of the cells was to be tested, the ingredients to be acted upon were added when the cells were first placed in the incubator; but when cell-free extracts were to be tested, the tissue treated as above described was allowed to undergo previous digestion for a variable period. The extract was then filtered off, thoroughly centrifugalised, and the clear extract was used for the experiment.

In the case of the pancreas and abdominal lymphatic glands, the tissue was first finely minced and subsequently treated in similar fashion to the intestinal mucosa.

The strength of extract employed was not the same in all the experiments, and is stated in each individual case.

The soap used was sodium oleate prepared from pure olive oil. The oleic acid obtained by hydrolysis from this soap had a melting point of 17°·5 C., and 0·214 grammes required 7·6 c.c. of $\frac{1}{10}$ N caustic soda for neutralisation, the theoretical amount being 7·57 c.c.

Series A.

Expt. 1.—Small intestine of cat during digestion of bread; no fat visible in lacteals, saline extract of 1 in 4, digested in incubator at 33° C. for 90 hours previous to addition of soap (2 per cent.) and glycerine (0·5 per cent.).

All the oleate dissolved, giving a clear solution; 1 hour later a few oily drops were visible in the solution under the microscope. Next morning (interval 17 hours 30 minutes) the fluid was yellow and cloudy like an emulsion, and some microscopic drops of fatty material were found floating on the surface of the fluid. Under the microscope a large number of oily globules of varying size were visible.

Expt. 2.—A like experiment on the abdominal lymphatic glands of the same animal, in saline extract of 1 in 5, same period of 90 hours previous digestion at 33° C. in incubator.

The extract, after centrifugalising, formed a clear reddish-yellow fluid in which no cellular elements were present when examined under the microscope; 10 c.c. of this fluid in a test-tube had 0·2 gramme of sodium oleate and 0·052 gramme of glycerine added, and the clear solution so obtained was heated in a water-bath to 38°·5 C.

The experiment was commenced at 6 P.M., and next morning at 10.30 A.M. (interval = 16 hours 30 minutes) there was a thick yellow oily layer on the top

* During most of the experiments chloroform was added to prevent bacterial growth, but to make certain that cellular activity was not inhibited by the presence of the chloroform, in certain experiments it was not added, and in these experiments the extractions were made by previously boiled salt solution or water.

which formed a temporary emulsion on shaking, and the fluid, examined under a low power of the microscope, showed a field thickly studded over with highly refractile globules, closely resembling milk, as seen under the microscope.

The oily layer was removed by shaking up with successive quantities of ether, in which it was readily soluble, and after evaporation of the ether, the amount of ethereal extractive was weighed, and the percentage of free oleic acid in it was determined by titration with decinormal sodic hydrate, in warm alcoholic solution, using rosolic acid as indicator. It was found that 87·5 per cent. of the ethereal extract consisted of free oleic acid.

The lymphatic extract used was alkaline to rosolic acid and remained so during the reaction.

Expt. 3.—This experiment shows that the production of free acid from soap is not stopped by the prolonged action of sulphuretted hydrogen upon the tissue cells or extracts.

Fresh ox lymphatic glands were treated as above described, and then extracted in a flask, with 5 volumes of distilled water, which was saturated with sulphuretted hydrogen gas, and then tightly corked and kept in an incubator at 36° C. for 92 hours.

At the end of the interval the contents still had a strong odour of sulphuretted hydrogen. The fluid was separated from the tissue elements, and a water clear extract of a greenish-brown colour was obtained.

The fluid was charged again with sulphuretted hydrogen and left at room temperature, tightly corked for another 10 days. It was then filtered from a slight deposit of sulphur, and found to be slightly acid (acidity = 0·24 N). It was rendered alkaline (alkalinity = 0·02 N to rosolic acid) by excess of sodium carbonate.

A portion of 40 c.c. had 0·8 gramme sodium oleate and 0·4 gramme of glycerine added; the flask was saturated with sulphuretted hydrogen, corked and placed in the incubator at 36° C. for 24 hours when an oily layer had appeared on the surface. A single extraction with ether gave a residue weighing 0·355 gramme and containing 0·341 gramme of free oleic acid.

Expt. 4.—Cell-free extracts of the pancreas and small intestine (mucosa) of three cats, taken in condition of inanition, prepared as before, and digested with normal saline for a period of 43 hours in an incubator at 36° C. The solutions were made distinctly alkaline to phenol-phthaleïn, and the strengths were made equal to 1 in 9 of the fresh tissues.

Each extract (intestine and pancreas) was then measured out into four portions, each of 25 c.c., and the two sets of solutions were treated as follows:—

No. 1.—25 c.c. extract + 0·5 gramme oleate + 0·1 gramme dextrose + 0·16 gramme glycerine.

No. 2.—25 c.c. extract + 0·5 gramme oleate + 0·16 gramme glycerine.

No. 3.—25 c.c. extract + 0·5 gramme oleate.

No. 4.—25 c.c. extract + 0·5 gramme oleate + boiling before digestion.

The eight tubes were then placed in a water-bath at 36° C. and examined at intervals.

Intestine.

An examination 1½ hours after the commencement of the experiment showed turbidity in all four of the intestinal extract tubes, and under the microscope thickly covered fields of oil globules.

The experiment was concluded at the end of 19 hours 35 minutes for analyses of the contents as given below, and at this time each tube showed a thick creamy layer, perhaps slightly less in No. 4 (boiled extract), but still very obvious. All four showed, under the microscope, fields crowded with oil globules of all sizes.

Pancreas.

This series of tubes, when examined 1 hour after the commencement of the experiment, showed in each case a milky fluid, and the microscopic examination demonstrated abundance of oil globules.

The reaction was obviously more intense than in the case of the intestine.

After the lapse of 18 hours from the commencement of the experiment, when it was stopped to make the analyses detailed below, there was a thick creamy layer at the top of each tube which was not apparently any less in quantity in No. 4 (boiled extract) than in any of the others.

The microscope showed in all four tubes oil globules of all sizes in great number.

The eight tubes were extracted with ether, and the weights of the ethereal extracts,* and the percentages of free oleic acid they contained, are given in the following table :—

Contents of tubes.	Weight of ethereal extract.	Weight of free oleic acid.	Percentage of free oleic acid.
	gramme.	gramme.	
Intestinal mucosa.			
No. 1. Oleate + glycerine + dextrose..	0·1800	0·1664	92·4
No. 2. Oleate + glycerine.....	0·1258	0·1256	99·8
No. 3. Oleate alone	0·1582	0·1466	92·7
No. 4. Oleate alone, then boiled	0·1322	0·1269	96·0
Pancreas.			
No. 1. Oleate + glycerine + dextrose..	0·2668	0·2580	96·7
No. 2. Oleate + glycerine	0·2568	0·2312	90·0
No. 3. Oleate alone	0·2176	0·2140	98·3
No. 4. Oleate alone, then boiled.....	0·2394	0·2284	95·4

This experiment shows that with cell-free extracts of pancreas and intestinal mucosa the main effect obtained is a conversion of sodium oleate into free oleic acid, and that there is no appreciable formation of olein.

Expt. 5. *Control Experiment.*—It might be argued that in the preceding experiments the soap was hydrolysed by the water or saline used as a solvent, and not by any active constituent derived from the tissues extracted, and accordingly a series of controls were arranged in which the same percentage of soap was dissolved, as follows :—

No. 1. Distilled water, 40 c.c. + sodium oleate, 0·8 gramme + glycerine, 0·4 gramme.

No. 2. Normal saline (0·75 per cent.), 40 c.c. + sodium oleate, 0·8 gramme + glycerine, 0·4 gramme.

No. 3. Solution of normal sodium carbonate (0·2 per cent.), 40 c.c. + saturation with carbon dioxide + glycerine, 0·4 gramme.

No. 4. Oxalated pig's plasma, 40 c.c. + sodium oleate, 0·8 gramme + glycerine, 0·4 gramme.

* The weighings throughout these experiments have been taken to $\frac{1}{5}$ milligramme. The figures represent fractions of a gramme.

The four solutions were placed in the incubator at 36° C., and examined as soon as they had attained the temperature of the incubator.

No. 1 was completely dissolved to a clear solution; Nos. 2 and 3 were opalescent and contained a good deal of undissolved oleate; No. 4 was clear but contained still a small amount of undissolved oleate.

Examined again, 4 hours later, the appearances presented by the four solutions were much the same as at the previous examination, while microscopic examination showed *no trace of fat globules in any of the four solutions*, but merely fine amorphous granules.

An examination made 46 hours after the commencement of the experiment, during the whole of which interval the solutions had been maintained at a temperature of 36° C., showed complete clear solution in Nos. 1 and 4 without a trace of cloudiness or precipitate, and giving a clear field under the microscope. Nos. 2 and 3 were opalescent and contained sediment, but there was no layer of oil and no globules visible under the microscope.

These controls demonstrate therefore that the formation of free oleic acid found in the previous experiments was due to some hydrolytic agent present in the tissue extracts.

Series B.

In the experiments of Series A, extracts free from cells were employed throughout, and as the results were different from those of previous observers who had employed the cells of the tissues, attention was now turned to similar experiments in the presence of the cells.

To make the experiments comparable with those of previous workers, the solutions, after digestion with the soap and glycerine in presence of the fresh cells, were evaporated down to dryness before extraction with ether for the determination of the nature of the fatty constituents.

By the use of this method the quantity of total ethereal extract is largely increased; but the control experiments, as well as the experiments recorded in Series C (*vide infra*), showed that the increase here obtained is due to dissolved soap and not to neutral fat.

Expt. 1.—The intestinal mucosa of a cat was prepared as already described, and two quantities of 6 grammes each were weighed out.

Portion No. 1 had 30 c.c. of normal saline, 0·6 gramme of sodium oleate, and 0·3 gramme of glycerine added; and portion No. 2, which was used as a control, had 30 c.c. of normal saline only added.

The two flasks containing the mixtures were placed in the incubator at 36° C. for a period of 43 hours, and were then taken out and the contents evaporated to dryness in porcelain basins on a steam bath.

When both were dry, 0·6 gramme of sodium oleate was added to No. 2, and then in each case four extractions were immediately commenced with ether. The united ethereal extracts in each case were evaporated to dryness, and the residues weighed.

Each residue was then dissolved in hot alcohol, and the amount of free fatty acid determined by titration with $\frac{1}{10}$ N caustic soda, using phenol-phthaleïn as indicator.

The results are given in the following table :—

	Weight of ethereal extract.	Weight of free oleic acid.	Difference.	Percentage of free oleic acid.
	gramme.	gramme.	gramme.	
No. 1.....	0·6290	0·4399	0·1891	69·9
No. 2 (control).....	0·3708	0·2510	0·1198	67·7

The free oleic acid obtained in No. 2 is derived from the neutral fat of the intestinal mucosa and not from the sodium oleate (compare Experiment 1, Series C). The difference between free oleic acid in No. 1 and No. 2 is due to hydrolysis of the sodium oleate in No. 1, and it is evident from the differences in column 3 that there is no appreciable synthesis of neutral fat. In fact, the difference lies in the opposite direction in Experiment 2 of this series (*vide infra*), proving that the 0·0693 gramme here apparently formed (0·1891—0·1198 gramme) lies within the limit of experimental error.

Expt. 2.—The abdominal lymphatics of the animal used in Experiment 1 were finely divided, and treated in similar fashion to the intestinal mucosa used in that experiment.

Portion No. 1 weighed 1·82 grammes, and to this were added 18 c.c. of normal saline, 0·36 gramme of sodium oleate, and 0·15 gramme of glycerine.

Portion No. 2 weighed 1·63 grammes, and to this were added 16 c.c. of normal saline only as a control. The two mixtures were digested at 36° C. for an interval of 115 hours, both were then evaporated to dryness, and 0·32 gramme of sodium oleate was added to No. 2. Each was then extracted four times with ether, the united ethereal extracts in each case were evaporated to dryness, and the weights of total ethereal extract and amounts of free oleic acid were determined.

The results were for ease of comparison calculated to 2 grammes of tissue, and are given in the following table : —

	Weight of ethereal extract.	Weight of free oleic acid.	Difference.	Percentage of free oleic acid.
	gramme.	gramme.	gramme.	
No. 1.....	0·4024	0·2751	0·1273	68·2*
No. 2.....	0·3782	0·1825	0·1957	48·2

Here the difference between total extract and free oleic acid is greater in the case of the control, showing that there is no synthesis of neutral fat.

Series C.

In this series of experiments, in addition to determining the amount of free oleic acid in the ethereal extract, the amount of neutral fat was

* The lower percentage of oleic acid obtained in this and in the preceding experiment is due to the dry method of extraction, in which more soap is dissolved out than in the wet extraction employed in Series A ; the difference is not due to neutral fat, but to soap, as is conclusively shown by the experiments in Series C.

also determined by Köttstorfer's saponification method (*vide supra*), and this amount was always found so low as to lie well within the limits of experimental error.*

To save repetition it may be stated that in each experiment of the series the extract, which in some cases was cell-free and in others contained the fresh tissue cells, was divided into four portions. Portion No. 1 had 2 per cent. of sodium oleate and 1 per cent. of glycerine added; No. 2 had 2 per cent. of sodium oleate alone; No. 3 had 2 per cent. of sodium oleate added after previous boiling; and No. 4 had nothing added, and was not boiled.

A comparison of the four sets of results after digestion will accordingly show the effects, if any, of presence of glycerine, as between No. 1 and No. 2; the effect of boiling upon the production of free oleic acid, as between Nos. 2 and 3; while No. 4 gives the amount of fatty extractives and action of digestion thereon in the tissues or extracts themselves.

The four portions were subjected to digestion for a stated period, which varied in the different experiments, and were next evaporated to dryness on a steam bath, after which each dry residue was treated in the following manner:—

Four extractions were made with ether, and the amount of residue on evaporation of the united ethereal extracts gave the total ethereal extractive.

The ethereal residue was dissolved in hot alcohol and titrated with decinormal sodic hydrate, using phenol-phthaleïn as indicator, titrating rapidly and taking the first appearance of a pink tinge as the end of the reaction, so as to avoid saponification of any trace of neutral fat which might be present. This gave a figure for the calculation of the amount of free oleic acid present.

After neutralisation, the alcoholic solution had a measured amount of a standardised approximately $\frac{1}{2}$N solution of alcoholic potash added, and was boiled in a flask fitted with a reflux tube for 20 minutes to half an hour. The solution was then titrated back to neutrality with standard $\frac{1}{2}$N hydrochloric acid, and the difference gave the amount of caustic potash used in saponifying and hence a figure for the determination of the amount of neutral olein present.

Expt. 1.—Intestinal mucosa cells (pig) in distilled water; period of digestion, 17 hours. In each portion were placed 10 grammes of intestinal mucosa and 30 c.c. of distilled water. No. 1 had added 0·8 gramme of sodium oleate and

* The figures given in the various experiments are intended merely to illustrate this point and not to form determinations of small actual amounts of neutral fat, the amount of alkali apparently required in saponification never exceeding 0·25 c.c. of standard alkali. The small amount used is also the only reason for the coincidences in value in the tables.

0·4 gramme of glycerine; No. 2, 0·8 gramme of sodium oleate only; No. 3, 0·8 gramme of sodium oleate after previous boiling; No. 4 had nothing added and was not boiled previous to digestion.

The results were as follows:—

	Total ethereal residue.	Free oleic acid.	Olein.	Soap, &c.
	gramme.	gramme.	gramme.	gramme.
No. 1.....	0·8034	0·1942	0·0294	0·5798
No. 2.....	0·6168	0·1885	0·0368	0·3915
No. 3.....	0·5772	0·1294	0·0368	0·4110
No. 4.....	0·0526	0·0309	0·0211	0·0016

It is here obvious, from inspection of the third column, that the amount of olein formed, as shown by the difference between No. 4, in the olein column, and the other three, is negligible and lies well within the limit of experimental error. Column No. 4 shows that the difference between total ethereal extract and free oleic acid is due to dissolved soap.

Addenda to this Experiment.

a. Controls with Distilled Water.

An experiment was next instituted to control the above and succeeding experiments of this series, by taking solutions in distilled water (*a*) of sodium oleate and glycerine, and (*b*) of sodium oleate alone, in identical strengths with those used above. The same amounts of solution, period of digestion, and modes of extracting and titrating were employed.

The following results were obtained:—

	Total ethereal extract.	Free oleic acid.	Olein.	Soap.
	gramme.	gramme.	gramme.	gramme.
No. 1 (oleate + glycerine)	0·6152	0·0704	0·0147	0·5301
No. 2 (oleate alone)....	0·5746	0·0760	0·0294	0·4692

This demonstrates that the chief thing dissolved and extracted in these controls was unaltered soap, and hence that the free oleic acid found in the case of the other experiments was not due to the experimental procedures employed, and further that the small amount of olein there found being no higher than here, was due to experimental error.

b. Action of the Intestinal Extract after Evaporation to Dryness and Extraction with Ether.

The experiment described above having shown (*vide* No. 3) that boiling, for 5 minutes, only somewhat diminished the power of the extract to convert sodium oleate into free oleic acid, and did not destroy it completely, the residue of No. 4, weighing 0·5786 gramme, to which no soap had been added, and which had now been not only boiled but reduced to dryness on the steam bath, and then subsequently exhausted with ether so that it contained no fat, was now tested by an additional experiment as to whether it still possessed any activity upon sodium oleate.

For this purpose it was treated with 40 c.c. of distilled water, and 0·8 gramme of sodium oleate was added to the mixture, which was then digested at 36° C. for a period of 17 hours; afterwards extraction was made with ether as before, and determinations made of total ethereal extract, free oleic acid, and olein with the following results:—

Total ethereal extract.	Oleic acid.	Olein.	Soap.
gramme. 0·5352	gramme. 0·2759	gramme. 0·0147	gramme. 0·2446

A comparison of the columns for oleic acid and soap with those obtained in the preceding control with distilled water only is sufficient to demonstrate that the activity of the extract, although somewhat impaired, is by no means destroyed by evaporation down to dryness at 100° C.

Expt. 2.—*Digestion with intestinal mucosa cells* (*pig*) *in distilled water for a shorter period* (*interval* 2½ *hours*).

The quantities and experimental procedures were as in Experiment 1, but the time of digestion was here reduced from 17 hours to 2½ hours, and the results were as follows:—

	Total ethereal extract.	Oleic acid.	Olein.	Soap.
	gramme.	gramme.	gramme.	gramme.
No. 1.....	0·5588	0·1664	0·0073	0·3851
No. 2.....	0·3956	0·1382	0·0147	0·2427
No. 3.....	0·5834	0·1382	0·0000	0·4452
No. 4.....	0·0432	0·0169	0·0294	0·0000*

The quantities of oleic acid here formed are almost as large as in the previous experiment, lasting 17 hours, showing that equilibrium had practically been attained in 2½ hours.

* In No. 4 the added oleic acid and olein slightly exceed the total ethereal extract, this is of course due to experimental error.

Expt. 3.—Intestinal mucosa cells (pig) in 0·75 per cent. saline, quantities and procedures as before, interval 2½ hours :—

	Total ethereal extract.	Oleic acid.	Olein.	Soap.
	gramme.	gramme.	gramme.	gramme.
No. 1.....	0·4620	0·1269	0·0147	0·3204
No. 2.....	0·3224	0·1269	0·0073	0·1882
No. 3.....	0·2706	0·0733	0·0147	0·1826
No. 4.....	0·0224	0·0197	0·0147	0·0000*

Expt. 4.—Clear intestinal mucosa extract (pig) in distilled water, 1 of gland to 5 of water; period of extraction = 44 hours; period of digestion = 2½ hours. Quantities taken and procedures as before :—

	Total ethereal extract.	Oleic acid.	Olein.	Soap.
	gramme.	gramme.	gramme.	gramme.
No. 1.....	0·3552	0·2171	0·0147	0·1234
No. 2.....	0·3338	0·2284	0·0220	0·0834
No. 3.....	0·3292	0·1916	0·0147	0·1199
No. 4.....	0·0302	0·0197	0·0220	0·0000

Expt. 5.—Clear intestinal mucosa extract (pig) in 0·75 per cent. saline, 1 of gland to 5 of saline; period of extraction = 44 hours; period of digestion = 2½ hours :—

	Total ethereal extract.	Oleic acid.	Olein.	Soap.
	gramme.	gramme.	gramme.	gramme.
No. 1.....	0·3252	0·1946	0·0073	0·1233
No. 2.....	0·3586	0·2171	0·0073	0·1342
No. 3.....	0·4774	0·2143	0·0147	0·2484
No. 4.....	0·0152	0·0085	0·0147	0·0000

Expt. 6.—Pancreatic cells (pig) in 0·75 per cent. saline, 1 of gland to 7 of saline; period of digestion = 15 hours. Quantities and procedures as before :—

	Total ethereal extract.	Oleic acid.	Olein.	Soap.
	gramme.	gramme.	gramme.	gramme.
No. 1.....	0·9330	0·6796	0·1323	0·1211
No. 2.....	0·9922	0·7360	0·1617	0·0945
No. 3.....	0·7518	0·2763	0·1941	0·2814
No. 4.....	0·3036	0·2058	0·0955	0·0023

* See *ante*.

The olein found in this experiment arises from the fat present in the pig's pancreas (compare Experiment 7). The amount in No. 4 is less indeed than in Nos. 1, 2, and 3, but this is most probably due to the protective action of the soap present which is attacked by the active substance of the gland.

Expt. 7.—Clear pancreatic extract (pig) in 0·75 per cent. saline, 1 of gland to 7 of saline; period of extraction = 23 hours; period of digestion = 16 hours:—

	Total ethereal extract.	Oleic acid.	Olein.	Soap.
	gramme.	gramme.	gramme.	gramme.
No. 1	0·5344	0·4766	0·0073	0·0505
No. 2	0·4582	0·4484	0·0073	0·0025
No. 3	0·5212	0·4596	0·0073	0·0543
No. 4	0·0396	0·0282	0·0147	0·0000

Expt. 8.—Abdominal lymphatic cells (pig) in 0·75 per cent. saline, 1 of gland to 7 of saline; period of digestion = 15 hours:—

	Total ethereal extract.	Oleic acid.	Olein.	Soap.
	gramme.	gramme.	gramme.	gramme.
No. 1	0·6738	0·2961	0·0073	0·3704
No. 2	0·5766	0·2933	0·0058	0·2775
No. 3	0·5062	0·1720	0·0080	0·3262
No. 4	0·0754	0·0296	0·0044	0·0414

Expt. 9.—Clear extract of abdominal lymphatic gland cells (pig) in 0·75 per cent. saline, 1 of gland to 7 of saline; period of extraction = 23 hours; period of digestion = 16 hours:—

	Total ethereal extract.	Oleic acid.	Olein.	Soap.
	gramme.	gramme.	gramme.	gramme.
No. 1	0·5382	0·4174	0·0294	0·0914
No. 2	0·4472	0·3722	0·0147	0·0603
No. 3	0·4612	0·3638	0·0294	0·0680
No. 4	0·0266	0·0282	0·0147	0·0000

Summary of Results and Conclusions.

1. Analyses of the lymph contained in the mesenteric lymphatic vessels during fat absorption demonstrate that at this stage of absorption practically all of the fatty constituents formed in the intestine during digestion have been re-synthesized into neutral fat.

This points to synthetic processes occuring in the cells present in the intestinal wall, and to the further conclusion that the cells of the mesenteric lymphatic glands are not normally concerned in the process of synthesis of the absorbed fatty constituents.

2. Analyses of the fatty constituents of the intestinal mucosa during fat absorption show a preponderance of neutral fat, but at the same time a considerable percentage of free fatty acid, showing that the synthesis is in progress, and has not obtained that completion found in the mesenteric lymphatic vessels.

3. No synthesis of neutral fat has been obtained from the normal cleavage constituents of fat (viz. soap and glycerine), by the action *in vitro* either of cells of the pancreas, intestinal mucosa or lymphatic glands, or of cell-free extracts of those tissues.

This observation taken in conjunction with the results above-mentioned proves that the living cell *in situ*, supplied with energy by the circulating blood, is capable of inducing a synthesis, which is not brought about by the detached cell or substances extracted from it.

A large number of similar synthetic actions of gland cells have been observed throughout the body, and this points to an important function of the cell as an energy transformer in such reactions as are endothermic in character, and require a supply of external energy. The action of a chemical catalyser or enzyme is simpler in character. The same enzyme cannot in any known case induce two different types of chemical transformation, one running exo-thermically and the other endo-thermically, and in so doing use up the energy re-acquired from the exo-thermic reaction. This is the essential difference between the chemical activity of the living cell when supplied by energy, and that of the enzyme, which in each specific instance is confined to a reaction of a single type.

An example is given by the transformation of carbohydrate into fat; here part of the carbohydrate is oxidised by the cell, and the energy obtained in this process is utilised for the conversion of another portion of the carbohydrate into fat with the taking up of energy. In most cases the reactions brought about, or increased in velocity, by enzymes are exo-thermic in character, giving rise to substances with less chemical energy than those from which they are formed, and in the few recorded cases of syntheses by enzymes, the products formed in the syntheses possess no measurably greater amount of energy than those from which they are formed.

The living cell from this point of view must be regarded as an energy transformer of much more complex type than the chemical catalyser or enzyme, and capable of producing, as shown by the present experiments, synthetic changes which do not occur as the result of the action of its chemical constituents when the complex structure of the cell is broken down, and its function as a whole abolished.

4. Extracts of pancreas, intestinal mucous membrane, and mesenteric lymphatic glands, possess the power of setting free oleic acid from solutions of sodium oleate.

The alkali split off from the sodium oleate becomes stably combined with some substance in the extracts and does not recombine with the oleic acid on evaporating down the solutions.

The power of setting free the oleic acid is diminished, but not destroyed, by boiling, nor even by evaporating the extracts to dryness.

The change occurs in faintly alkaline solution and is completed without the reaction becoming acid.

No similar change is obtained under the conditions of concentration of the experiment with water or saline solution, and hence the reaction is due to some substance in the extracts.

Such a change may possibly in the cell be the initial change which the soap undergoes in the synthesis into neutral fat.

The change obviously will act as a protection to the cells of the body against invasion by poisonous soaps in the circulation, and here serves a similar function to that seen in the conversion of the albumoses into coagulable proteids by the intestinal cells.